ELECTRON SPIN RESONANCE OF COPPER LABELLED MYOGLOBIN CRYSTALS

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1. Introduction

The interest in biological role of the transition metal ions [1–3] has lead to various studies of copper–myoglobin and copper–hemoglobin complexes [4–7]. All the ESR work in these complexes has been done in frozen solutions with the concomitant loss of information which comes from the studies of anisotropic properties of the transition metal ions [8–11].

We report here on an ESR study of single crystals of sperm whale met–myoglobin doped with Cu^{2+} ions (Mb:Cu^{2+}).

The choice of this particular complex has been motivated by the following:

a) The knowledge of the tridimensional structure of myoglobin [12] and the knowledge of its crystalline structure [13]. The crystals are monoclinic with the $\vec{c}$ axis making an angle of 105.5° with the other axes. The unit cell contains two non-equivalent molecules.

b) The indication of the molecular site of the cupric ion from X-ray diffraction work of Banaszak [14]; this study situates the ion site near asparagine GH4, lysine A14, and the imidazole ring of histidine A10. It seems clear that the copper ion is bound to a nitrogen of the imidazole ring of histidine A10.

c) The existence of several studies [15–19], using different chemical and physical methods, of Mb:Cu^{2+} and Mb:Zn^{2+} complexes.

We have used sperm whale met–myoglobin (Sigma Co.) and grew single crystals according to Kendrew and Parrich's method [13]. The crystals, typically 5 mm × 3 mm × 2 mm were doped with Cu^{2+} by immersion in a saturated solution of ammonium sulphate containing diluted $CuSO_4$ and heating slightly the ensemble. The solution has a 80:1 excess of Cu^{2+} ions with respect to the myoglobin concentration. Experiments were performed at 77°K and at room temperature using a Varian Spectrometer V-4502.

The sample was oriented in the cavity making use of the *ab* crystalline face and of the well known anisotropic Fe^{3+} signal [8] Angular variation of the spectra were measured in the planes: *ab*, *ac** (*c** is an axis perpendicular to the *ab* plane) and *bc**.

For an arbitrary orientation of the magnetic fields with respect to the crystalline axes we observe, besides the Fe^{3+} ESR signal, a pair of four nearly equidistant lines. These are due to two magnetically inequivalent Cu^{2+} ions ($S = \frac{1}{2}$) each one with its hyperfine quadruplet ($I_{Cu} = \frac{3}{2}$) (fig.1). The angular variation of each spectrum is described by the spin-Hamiltonian:

$$\mathcal{H} = \mu_B \vec{H} \cdot \tilde{g} \cdot \vec{S} + \vec{S} \cdot \tilde{A} \cdot \vec{I} \qquad (1)$$

which gives, in a second order perturbation calculation [20] for the case of axial Zeeman and hyperfine interactions, the relation between the magnetic fields and a microwave frequency:

$$h\nu = g\,\mu_B\,H + KM_I + \frac{A_\perp^2\,(A_{/\!/}^2 + K^2)\,[I\,(I+1) - M_I^2]}{4\,K^2\,g} + \frac{(A_{/\!/}^2 - A_\perp^2)^2\,g_{/\!/}^2\,g_\perp^2\,\sin^2\theta\,\cos^2\theta}{2\,g^5\,K^2}\,M_I^2 \qquad (2)$$

where

$$g^2 = g_{/\!/}^2 \cos\theta + g_\perp^2 \sin^2\theta$$

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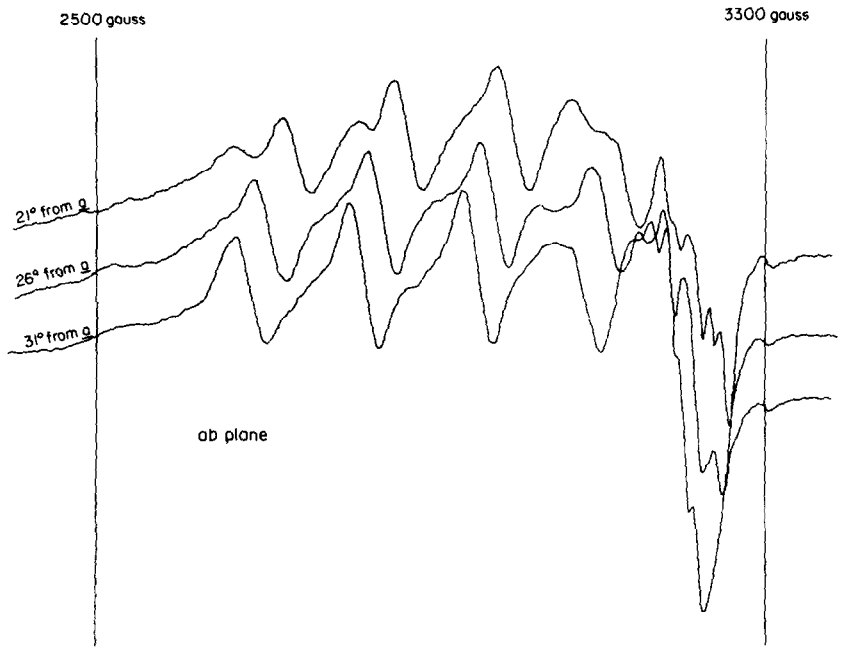

Fig.1. Examples of Cu hyperfine ESR spectra for three arbitrary orientations in the *ab* plane.

and

$$g^2 K^2 = g_{/\!/}^2 A_{/\!/}^2 \cos^2\theta + g_\perp^2 + A_\perp^2 \sin^2\theta$$

From the analysis of the angular variation of the spectra in the three above mentioned planes we conclude that:

a) The $\hat{g}$ and **A** tensors are axially symmetric in the same principal system;

b) The principal axes of these tensors for the two Cu^{2+} ions lie in the *ab* plane and make the same angle α with the *b* direction (see fig.2); this is indicated by the fact that in both *ac** and *bc** planes the two spectra are superposed (the two Cu^{2+} ions are then magnetically equivalent) (see fig.3);

c) The value of the angle α can be established from the angular variation in the *ab* plane (fig.2) measuring the angular separation between the two low-fields extremes of the spectra; $\alpha = 39^\circ \pm 2^\circ$ -a slight disorientation of the crystal limits the accuracy of α and of the spin-Hamiltonian parameters. The line width of the copper ESR spectra is about 55 gauss also contributing to the error.

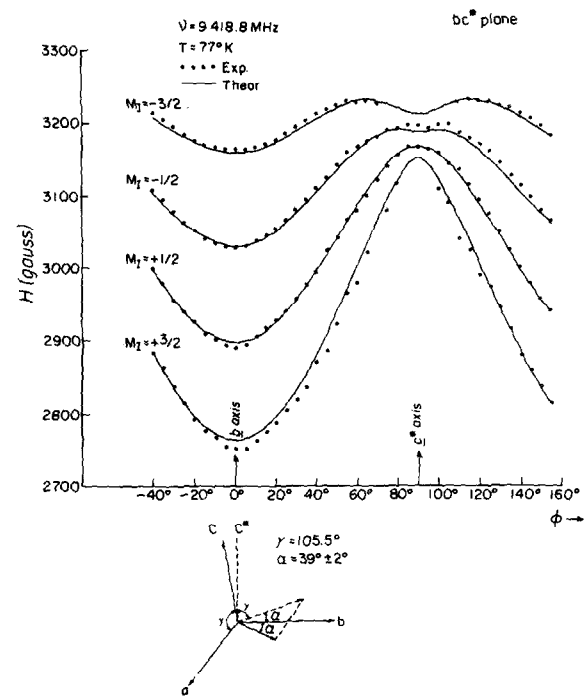

Fig.2. Position of the ESR lines as a function of angle in the *bc** plane.

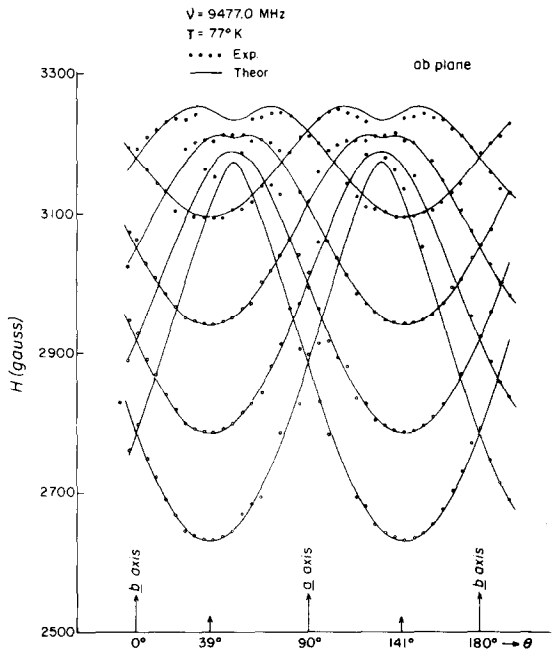

Fig.3. Position of the ESR lines as a function of angle in the *ab* plane.

The spin-Hamiltonian parameters were obtained by fitting the theoretical curves (eq. 2) to the experimental angular variations. Their values are:

$$g_{/\!/} = 2.328 \pm 0.002;\ g_{\perp} = 2.069 \pm 0.002$$

$$\left| \frac{A_{/\!/}}{g_{/\!/}\,\mu_B} \right| = (162 \pm 3) \text{ gauss};\quad \left| \frac{A_{\perp}}{g_{\perp}\,\mu_B} \right| = (20 \pm 3) \text{ gauss}$$

As neither the plane ac^* nor the plane bc^* is a symmetric plane of the crystalline structure we can conclude that the magnetic equivalence of the two coppers when $\vec{H}$ is parallel to these planes is accidental. Moreover we consider that each copper ion is bonded to each of the two non-equivalent myoglobin molecules of the unit cell.

Another interesting feature of the spectra is that when $\vec{H}$ is nearly parallel to the 'perpendicular' direction (axes associated with g_{xx} or g_{yy}), hyperfine lines corresponding to $M_I = -{}^3/_2$ and $M_I = -{}^1/_2$ are split in three components with maximum separation equal to 17 gauss. This splitting may be attributed to a superhyperfine magnetic interaction of the unpaired electronic spin with the nucleus of a neighbouring nitrogen (I = 1). This feature agrees with results obtained by Gurd et al. [19] and confirms the X-ray diffraction work of Banaszak et al. [14] which indicates that there exists a preferential bonding of the metal ion to a nitrogen of the imidazole ring of the histidine A10 in $Mb{:}Cu^{2+}$.

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